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A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans

Lê, K A ; Faeh, D ; Stettler, R ; Ith, M ; Kreis, R ; Vermathen, P ; Boesch, C ; Ravussin, E ; Tappy, L

Abstract: BACKGROUND: High fructose consumption is suspected to be causally linked to the epidemics of obesity and metabolic disorders. In rodents, fructose leads to insulin resistance and ectopic lipid deposition. In humans, the effects of fructose on insulin sensitivity remain debated, whereas its effect on ectopic lipids has never been investigated. **OBJECTIVE:** We assessed the effect of moderate fructose supplementation on insulin sensitivity (IS) and ectopic lipids in healthy male volunteers (n = 7). **DESIGN:** IS, intrahepatocellular lipids (IHCL), and intramyocellular lipids (IMCL) were measured before and after 1 and 4 wk of a high-fructose diet containing 1.5 g fructose . kg body wt(-1) . d(-1). Adipose tissue IS was evaluated from nonesterified fatty acid suppression, hepatic IS from suppression of hepatic glucose output (6,6-2H2-glucose), and muscle IS from the whole-body glucose disposal rate during a 2-step hyperinsulinemic euglycemic clamp. IHCL and IMCL were measured by 1H magnetic resonance spectroscopy. **RESULTS:** Fructose caused significant (P < 0.05) increases in fasting plasma concentrations of triacylglycerol (36%), VLDL-triacylglycerol (72%), lactate (49%), glucose (5.5%), and leptin (48%) without any significant changes in body weight, IHCL, IMCL, or IS. IHCL were negatively correlated with triacylglycerol after 4 wk of the high-fructose diet (r = -0.78, P < 0.05). **CONCLUSION:** Moderate fructose supplementation over 4 wk increases plasma triacylglycerol and glucose concentrations without causing ectopic lipid deposition or insulin resistance in healthy humans.

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A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans^{1–4}

Kim-Anne Lê, David Faeh, Rodrigue Stettler, Michael Ith, Roland Kreis, Peter Vermaaten, Chris Boesch, Eric Ravussin, and Luc Tappy

ABSTRACT

Background: High fructose consumption is suspected to be causally linked to the epidemics of obesity and metabolic disorders. In rodents, fructose leads to insulin resistance and ectopic lipid deposition. In humans, the effects of fructose on insulin sensitivity remain debated, whereas its effect on ectopic lipids has never been investigated.

Objective: We assessed the effect of moderate fructose supplementation on insulin sensitivity (IS) and ectopic lipids in healthy male volunteers ($n = 7$).

Design: IS, intrahepatocellular lipids (IHCL), and intramyocellular lipids (IMCL) were measured before and after 1 and 4 wk of a high-fructose diet containing 1.5 g fructose · kg body wt⁻¹ · d⁻¹. Adipose tissue IS was evaluated from nonesterified fatty acid suppression, hepatic IS from suppression of hepatic glucose output (6,6-²H₂-glucose), and muscle IS from the whole-body glucose disposal rate during a 2-step hyperinsulinemic euglycemic clamp. IHCL and IMCL were measured by ¹H magnetic resonance spectroscopy.

Results: Fructose caused significant ($P < 0.05$) increases in fasting plasma concentrations of triacylglycerol (36%), VLDL-triacylglycerol (72%), lactate (49%), glucose (5.5%), and leptin (48%) without any significant changes in body weight, IHCL, IMCL, or IS. IHCL were negatively correlated with triacylglycerol after 4 wk of the high-fructose diet ($r = -0.78$, $P < 0.05$).

Conclusion: Moderate fructose supplementation over 4 wk increases plasma triacylglycerol and glucose concentrations without causing ectopic lipid deposition or insulin resistance in healthy humans. *Am J Clin Nutr* 2006;84:1374–9.

KEY WORDS Cardiovascular disease risk, dyslipidemia, healthy men, ectopic lipids, insulin sensitivity, fructose

INTRODUCTION

Over the past decades, fructose consumption per capita has dramatically increased, mainly because of a higher consumption of sugar-sweetened beverages (1). Furthermore, several authors have suggested that fructose (as either sucrose or high-fructose corn syrup) might play a role in the onset of metabolic disorders and excess weight gain (1, 2). This concern is supported by observations that, in rodents, a high-fructose diet (HFD) induces hepatic insulin resistance, increases intrahepatocellular lipids (IHCL), and stimulates hepatic de novo lipogenesis within a few days (3, 4). When sustained over longer periods of time, high fructose or sucrose intakes induce hepatic steatosis (5, 6) and

whole-body insulin resistance with a concomitant accumulation of intramyocellular lipids (IMCL) (3, 7, 8). In humans, several studies have addressed the chronic effects of fructose ingestion on lipid and carbohydrate metabolism, but many issues remain unresolved. A high fructose intake has clearly been shown to increase plasma triacylglycerol concentrations (9–11). In contrast, the effect of high fructose intakes on insulin sensitivity is still debated. As recently reviewed, some authors have observed increased fasting glucose and insulin concentrations after high fructose consumption, whereas others have reported no effect on glucose homeostasis or markers of insulin sensitivity (12). No study to date had addressed the effects of dietary fructose on ectopic lipids.

In a previous study, we reported that a 6-d HFD induces dyslipidemia as well as hepatic and adipose tissue insulin resistance without altering whole-body insulin sensitivity (13). To further delineate the metabolic consequences of a longer period of high fructose consumption with a focus on insulin sensitivity, we studied a group of 7 healthy males submitted to a 4-wk HFD. The amount of daily fructose consumption added to the diet corresponded with the fructose content of 2 L of soda.

SUBJECTS AND METHODS

Subjects

Seven healthy, nonsmoking, white male volunteers (mean \pm SEM age: 24.7 \pm 1.3 y) took part in the study (Table 1). According to a physical examination and a brief medical history, all

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TABLE 1Fasting anthropometric and metabolic variables¹

	Baseline	High-fructose diet			
		1 wk	2 wk	3 wk	4 wk
Anthropometric variables					
Body weight (kg)	69.3 ± 2.6	69.3 ± 2.5	69.0 ± 2.6	69.3 ± 2.7	69.5 ± 2.7
Body fat (%)	17 ± 1	17 ± 1	16 ± 1	16 ± 1	16. ± 1
Mean blood pressure (mm Hg)	83 ± 2	83 ± 2	84 ± 1	85 ± 2	84 ± 1
Metabolic variables					
Glucose (mmol/L)	4.9 ± 0.1	5.0 ± 0.1	5.2 ± 0.1	4.9 ± 0.1	5.2 ± 0.1 ²
Lactate (mmol/L)	0.83 ± 0.05	0.97 ± 0.11	1.24 ± 0.11 ²	1.25 ± 0.06 ²	0.92 ± 0.04
Insulin (pmol/mL)	50.4 ± 3.6	57.6 ± 5.4	56.4 ± 5.4	58.8 ± 5.4	51.0 ± 2.4
Glucagon (pmol/L)	67 ± 9	67 ± 8	70 ± 10	70 ± 10	71 ± 11
Total triacylglycerols (mmol/L)	0.64 ± 0.05	0.87 ± 0.08 ²	0.83 ± 0.05 ²	0.76 ± 0.07	0.94 ± 0.06 ²
VLDL-triacylglycerols (mmol/L)	0.36 ± 0.04	0.62 ± 0.08 ²	0.58 ± 0.05 ²	0.53 ± 0.07 ²	0.70 ± 0.06 ²
Nonesterified fatty acids (μmol/L)	740 ± 92	488 ± 37 ²	368 ± 17 ²	378 ± 45	515 ± 39 ²
Cholesterol (mmol/L)	3.8 ± 0.3	3.8 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	4.0 ± 0.3
β-Hydroxybutyrate (mmol/L)	0.24 ± 0.04	0.12 ± 0.04	0.06 ± 0.02 ²	0.06 ± 0.02 ²	0.05 ± 0.01 ²
Leptin (ng/mL)	2.1 ± 0.2	2.3 ± 0.2 ²	2.5 ± 0.3	3.3 ± 0.4 ²	3.2 ± 0.4 ²

¹ All values are $\bar{x} \pm \text{SEM}$; $n = 7$.² Significantly different from baseline, $P < 0.05$ (Friedman's ANOVA and Page's test, followed by Wilcoxon's matched-pairs signed-ranks test).

subjects were in good health, with body mass indexes (in kg/m^2) between 19 and 25, and were moderately physically active (<1 h/wk). They were not taking any medications and did not regularly consume alcohol or sugar-sweetened beverages. The study was approved by the ethical committee of Lausanne University School of Medicine, and all participants provided written informed consent.

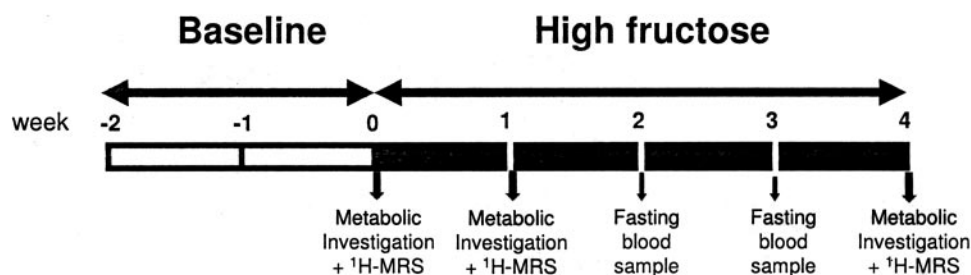
Study design and diet

During the initial 2 wk of the study, all subjects were thoroughly instructed by a trained dietitian to consume an isoenergetic diet containing $\approx 55\%$ carbohydrates, 30% fat, and 15% protein. They were further instructed to consume a minimal amount of either sucrose-sweetened or artificially sweetened drinks and food during this period, with a resultant fructose consumption of <20 g/d. Thereafter, they were switched to a HFD consisting of the same isoenergetic diet with the addition of $1.5 \text{ g fructose} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ for 4 wk. Fructose was consumed as a 20% solution with the 3 main meals and represented an excess of 18% of the subjects' daily energy requirement. The subjects' adherence to the prescribed fructose consumption was verified by collecting the empty fructose containers, and all subjects reported a 3-d dietary record before each test. Leisure-time sport activity was restricted to <1 h/wk throughout the study period. Fasting blood samples were collected weekly, and insulin sensitivity, IHCL, and IMCL were

measured at baseline and after 1 and 4 wk of the HFD (**Figure 1**). Insulin sensitivity was determined by a two-step hyperinsulinemic euglycemic clamp. IHCL and IMCL contents were assessed by proton magnetic resonance spectroscopy ($^1\text{H-MRS}$).

Metabolic investigation

Subjects reported at 0700 to the metabolic unit of the Lausanne University Hospital after they had fasted for 12 h overnight. On arrival, the subjects were asked to void, and their body composition was estimated from subcutaneous skinfold-thickness measurements at the biceps, triceps, subscapular, and suprailiac sites (14). While the subjects rested quietly in a bed in a semirecumbent position, an indwelling catheter was inserted into the vein of the right wrist for blood sampling. A second indwelling catheter was inserted into an antecubital vein of the other arm for glucose, insulin, and tracer infusions. Whole-body glucose turnover was assessed in the basal condition after a 2-h $6,6\text{-}^2\text{H}_2$ glucose infusion (bolus: 2 mg/kg ; continuous: $20 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Blood was collected at baseline for measurement of plasma concentrations of glucose, insulin, glucagon, leptin, lactate, nonesterified fatty acids (NEFAs), β -hydroxybutyric acid, triacylglycerol, total cholesterol, and VLDL, LDL, and HDL subfractions. Energy expenditure and substrate utilization were continuously measured by indirect calorimetry (ventilated canopy) from 0800 to 1300 (15) by using the equations of Livesey and Elia (16). At

**FIGURE 1.** Experimental protocol. MRS, magnetic resonance spectroscopy.

baseline, heart rate and blood pressure were measured 3 times over a 20-min period.

Whole-body, liver, and adipose insulin sensitivity were measured for 3 h after the initial 2-h tracer infusion, from 1000 to 1300. A two-step, hyperinsulinemic, euglycemic clamp (0.3 and $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, 90 min each) (17, 18) was performed in combination with measures of hepatic glucose output ($6,6\text{-}^2\text{H}_2$ glucose, "hot infusion model") (19) and lipolysis (plasma NEFA concentrations). Blood samples were collected every 5 min during the clamp to monitor plasma glucose concentrations and at 30-min intervals for the analysis of tracer, insulin, glucagon, triacylglycerol, and NEFA concentrations.

Analytic procedures

Plasma was immediately separated from blood by centrifugation at 4°C for 10 min at $3600 \times g$ and was stored at -20°C . Colorimetric methods were used to assess plasma concentrations of NEFAs (kit from Wako Chemicals, Freiburg, Germany) and triacylglycerol (kit from Biomérieux Vitex Inc, Lyon, France). Commercial radioimmunoassay kits were used for the determination of plasma insulin, glucagon, and leptin (LINCO Research, St Charles, MO). Subfractions of lipoproteins were separated by ultracentrifugation. β -Hydroxybutyric acid and lactate concentrations were measured enzymatically by using kits from Boehringer (Boehringer Mannheim, Mannheim, Germany). During the clamp, plasma glucose concentrations were measured by the glucose oxidase method with a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma $6,6\text{-}^2\text{H}_2$ glucose isotopic enrichment was measured by gas chromatography-mass spectrometry (Hewlett-Packard Instruments, Palo Alto, CA), as previously described (20).

^1H Magnetic resonance spectroscopy

All ^1H -MRS examinations were performed on a clinical 1.5 T MR scanner (Signa; General Electric Medical Systems, Waukesha WI) by using a transmit-receive extremity coil for the calf and a flexible receive coil in combination with the body transmit coil for the liver. Data acquisition and processing of spectra from the tibialis anterior followed the previously described protocol (21). In short: single-voxel ^1H -MR spectra were acquired with an optimized point-resolved spectroscopy (PRESS) sequence (repetition time = 3 s, echo time = 20 ms, 128 acquisitions, 16 phase rotating steps, 2-kHz bandwidth, 1024 points). The voxel in the calf measured $11 \times 12 \times 18 \text{ mm}^3$ in R/L \times A/P \times I/S direction and was placed in the tibialis anterior muscle of the right leg. The spectra were quantified after eddy current correction by using the fully relaxed unsuppressed water signal as internal concentration standard. All results are expressed as mmol/kg wet wt.

Acquisition of reproducible and reliable MR spectra from a volume in the liver is hampered by effects of respiratory and potentially cardiac motion. To reduce these influences, data acquisition was double-triggered to both respiration and the electrocardiogram by using a method developed for ^1H -MRS of the heart (22), which makes use of the fact that the electrocardiogram amplitude depends on respiratory motion and that allows us to restrict electrocardiogram-triggered data acquisition to periods of expiration. On the basis of axial MR images (spoiled gradient recalled echo sequence, 60° flip angle, 1.5-ms echo time, 0.11-s repetition time, 4-mm slice thickness, 1-mm gap between slices, 40-cm field of view, 512×192 matrix size) obtained in breath

hold, a volume of interest of 4.3 cm^3 was placed in a lateral area of the liver and repositioned at the same location in follow-up examinations. MR spectra were recorded from this volume with the same short echo time PRESS sequence used for the investigation of the calf (echo time 20 ms). Sixty-four acquisitions with water presaturation were recorded and stored individually for each spectrum. Effects of residual motion were found to be evidenced by small shifts in resonance frequency. They were accounted for by realigning those individual scans in a frequency domain that fell into a bandwidth of 12 Hz and by discarding those acquisitions with a lipid peak shifted by more than $\pm 6\text{ Hz}$. Spectra were processed, fitted, and quantitated similarly to the muscle spectra. Quantitation to obtain IHCL in units of mmol/kg was based on the median water signal from 8 separate acquisitions obtained without water suppression, a T_2 of 50 ms for this water signal (as determined from separate acquisitions with varying echo time), and an assumed liver water content of 71.1%. Reproducibility of IMCL and IHCL determinations on immediate repetition of the measurement in completely independent examinations was established earlier and was found to be 6% for IMCL (23) and 10% for IHCL (R Kreis, P Vermathen, M Ith, K-A Lê, L Tappy, C Boesch, unpublished observations, 2006), whereas variations between healthy subjects were found to be much larger.

Statistical analysis

Throughout the manuscript, data are expressed as means \pm SEMs. Statistical analyses were performed with STATA version 8.2 (Stata Corp, College Station, TX), and $P < 0.05$ was considered statistically significant. The significance of mean differences among conditions was determined with Friedman's analysis of variance (ANOVA) and Page's test (24). Post hoc comparisons were done by using the Wilcoxon's matched-pairs signed-ranks test. Relations between IHCL and IMCL and total triacylglycerol or VLDL-triacylglycerol were assessed by using Spearman's correlation test.

RESULTS

Body weight, body composition, and mean blood pressure were not significantly affected by the 4-wk HFD intervention. However, within one week, the HFD caused significant ($P < 0.05$) increases in fasting VLDL-triacylglycerol (72%), total triacylglycerols (36%), and leptin (48%), whereas lactate increased only after 2 wk (49%) and glucose after 4 wk (5.5%). Fasting NEFA and β -hydroxybutyrate concentrations decreased significantly after 1 and 2 wk of the HFD, respectively (NEFA: -34% ; β -hydroxybutyrate: -75%). Fasting insulin, glucagon, and total cholesterol concentrations did not change significantly during the HFD ($P > 0.05$; Table 1 and Figure 2).

Despite no significant changes in energy expenditure and fasting hepatic glucose output over the 4-wk HFD treatment, carbohydrate oxidation increased after 1 wk (34%; $P < 0.05$) with a concomitant trend toward decreased lipid oxidation ($P = 0.09$; Table 2). IMCL and IHCL concentrations were unchanged by HFD treatment (Figure 2). There was a significant negative correlation between IHCL and fasting triacylglycerol after 4 wk of the HFD ($r = -0.78$, $P < 0.05$), whereas this correlation fell short of significance at baseline and after 1 wk ($r = -0.55$ and -0.54 , respectively; $P > 0.05$). No significant correlation was observed between IMCL and total- or VLDL-triacylglycerol.



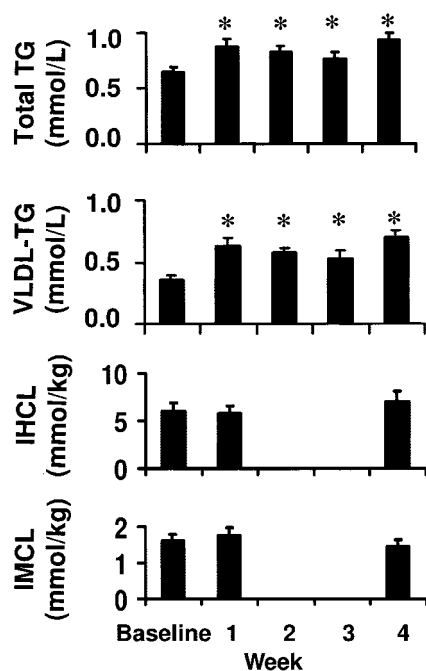


FIGURE 2. Mean (\pm SEM) plasma triacylglycerol (TG), VLDL-TG, intrahepatocellular lipids (IHCL), and intramyocellular lipids (IMCL) in response to the high-fructose diet. $n = 7$. *Significantly different from baseline, $P < 0.05$ (Friedman's ANOVA and Page's test, followed by Wilcoxon's matched-pairs signed-ranks test).

During the hyperinsulinemic clamp studies, plasma glucose concentrations were successfully maintained at 5.5 ± 0.1 mmol/L. During the baseline clamp procedure, insulin concentrations were 222 ± 42 pmol/L during the first step and increased to 510 ± 12 pmol/L during the second step of the clamp, reaching similar plateaus after 1 and 4 wk of the HFD. The suppression of hepatic glucose output by the low-dose insulin infusion ($t = 90$ min) was not significantly affected by the HFD treatment. Similarly, the glucose disposal rate, glucose and lipid oxidation rates, and the nonoxidative glucose disposal rate ($t = 180$ min) were not significantly affected by the HFD treatment (Table 2).

DISCUSSION

A 4-wk fructose supplementation ($1.5 \text{ g} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$) induced moderate but sustained increases in plasma triacylglycerol, VLDL-triacylglycerol, and leptin paralleled by a modest increase in fasting plasma glucose without any significant change in body weight. In contrast, hepatic, adipose, and whole-body insulin sensitivity as well as liver and muscle lipid contents were unchanged by the 4-wk fructose supplementation.

The increase in fasting VLDL-triacylglycerol observed in the present study corroborates several previous reports in humans (10, 11, 25) and animals (26). Both a stimulation of hepatic VLDL-triacylglycerol synthesis and secretion and decreased VLDL-triacylglycerol clearance may be involved in this process (27).

In a previous study, we reported that fructose supplementation at twice the dose used in the present study led to an 80% increase in plasma VLDL-triacylglycerol (13). Our present findings further extend this observation by showing that this effect, first, is dependent on the dose of fructose administered, and second, is

TABLE 2

Metabolic variables under basal and hyperinsulinemic (T90, low insulin infusion; T180, high insulin infusion) conditions¹

	Baseline	High-fructose diet	
		1 wk	4 wk
Hepatic glucose output ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)			
Baseline	13.3 ± 0.5	13.9 ± 0.5	13.9 ± 0.5
T90	6.1 ± 0.5	7.8 ± 0.5	6.1 ± 1.1
T180	3.9 ± 2.2	2.2 ± 2.2	1.7 ± 2.2
Glucose disposal rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)			
T90	18.3 ± 0.5	20.6 ± 0.5	19.4 ± 1.1
T180	28.3 ± 3.4	30.0 ± 4.4	28.3 ± 4.4
Carbohydrate oxidation ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)			
Baseline	8.3 ± 1.7	11.1 ± 1.1^2	9.5 ± 1.7
T90	11.1 ± 1.1	10.6 ± 1.1	12.8 ± 2.2
T180	13.9 ± 1.1	18.9 ± 1.7^2	17.2 ± 1.7
Nonoxidized carbohydrates ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)			
Baseline	5.0 ± 1.7	3.3 ± 1.7	5.0 ± 2.2
T90	7.2 ± 0.5	10.0 ± 1.1^2	7.2 ± 2.2
T180	16.1 ± 3.9	13.3 ± 5.0	12.8 ± 5.6
Lipid oxidation ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)			
Baseline	0.7 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
T90	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
T180	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1^2
Energy expenditure (kcal/min)			
Baseline	1.02 ± 0.04	0.98 ± 0.02	0.98 ± 0.03
T180	1.04 ± 1.01	1.01 ± 0.02	1.03 ± 0.03

¹ All data are $\bar{x} \pm \text{SEM}$; $n = 7$.

² Significantly different from baseline, $P < 0.05$ (Wilcoxon's matched-pairs signed-ranks test).

sustained for ≥ 4 wk. Given the epidemiologic association observed between plasma total or VLDL-triacylglycerol concentrations and atherosclerotic vascular disorders (28), these observations strongly suggest that consumption of even modest amounts of fructose may significantly increase the risk of development of cardiovascular disease.

We also observed a small but significant increase in fasting glycemia after 4 wk of the HFD. In our previous study, supplementation with twice the dose of fructose used in this study led to significant increases in fasting glycemia and hepatic glucose output (13). Our present results failed to reproduce the increase in hepatic glucose output, probably because of the lower fructose intake. However, our observation of a higher fasting plasma glucose concentration still suggests some degree of impairment in the inhibition of glucose production by glycemia (29, 30).

These results contrast with those obtained in rodents submitted to a high-sucrose diet. After sucrose feeding, rodents develop early hepatic insulin resistance and increased IHCL. High sucrose intake eventually leads to the development of whole-body insulin resistance with a concomitant increase in IMCL after 5 to 6 wk of treatment (3). The fructose part of sucrose appears to be the major culprit for these effects (31). This time course in the development of insulin resistance suggests a central role of the liver before the development of whole-body insulin resistance


(32). The metabolic events observed in our study differ markedly from these animal studies. First, neither IHCL nor IMCL increased at any time; second, except for the modest increase in glycemia observed after 4 wk, there was no evidence for a reduction in hepatic or whole-body insulin sensitivity. Several explanations may account for these differences. The relative doses of fructose consumed in these animal experiments were much larger than in the present study. It has also been reported that, in rodents, lower sucrose intake as well leads to the development of insulin resistance, although with a longer delay (33). It is therefore possible that such modest doses of fructose would require a longer exposure to induce adverse effects on insulin sensitivity.

The absence of any change in IHCL and IMCL despite a sustained increase in plasma triacylglycerol and VLDL-triacylglycerol was unexpected and is most likely related to the absence of insulin resistance after the HFD. Fructose, by increasing *de novo* lipogenesis, may enhance hepatic triacylglycerol production. These newly formed hepatic triacylglycerols can in turn be secreted as VLDL-triacylglycerol, stored as IHCL, or oxidized. If hepatic triacylglycerol synthesis were to determine both VLDL-triacylglycerol concentration and IHCL deposition, one would expect a positive correlation between IHCL and VLDL-triacylglycerol after fructose-induced stimulation of triacylglycerol synthesis. Our results are clearly at odds with this scenario. We observed a significant negative correlation between IHCL and total or VLDL-triacylglycerol after 4 wk of the HFD. This indicates that the HFD increased plasma triacylglycerol without altering IHCL, and this observation supports the hypothesis that exportation of newly formed triacylglycerol as VLDL-triacylglycerol is a key element to prevent IHCL accumulation. The HFD failed to increase not only IHCL but also IMCL and failed to alter whole-body insulin sensitivity. Therefore, we postulate that the extra amount of triacylglycerol formed from fructose was essentially secreted as VLDL, thus preventing ectopic fat deposition in the liver.

Finally, high fructose feeding led to a continuous rise in fasting plasma leptin concentrations. This observation is consistent with experiments performed on isolated adipocytes showing that fructose and glycolytic substrates and metabolites increase leptin secretion (34) and with studies performed in rats fed a high-fructose diet (35). In the present study, both fructose-induced hyperlactatemia and hypertriglyceridemia may therefore have contributed to stimulating leptin secretion by adipocytes. The increased leptin concentration may in turn account for the absence of body weight gain despite the substantial fructose energy load added to the diet. Indeed, it is possible that a reduction of nonfructose nutrients occurred, in which hyperleptinemia may play a role.

We want to stress that our study has several limitations that must be kept in mind when interpreting the results. First, because it was performed as an outpatient study, it was not possible to assess compliance with the dietary prescription and fructose supplementation. However, the consistent increases in plasma triacylglycerol concentrations and basal carbohydrate oxidation observed after the HFD make us confident that the bulk of fructose was indeed consumed. Second, the study was designed as an uncontrolled study and included a baseline with a low-fructose diet followed by an isocaloric diet with fructose supplementation. Because no comparison was made with a glucose-supplemented diet, it remains possible that the effects observed

were due to the increased carbohydrate intake rather than to specific effects of fructose. Furthermore, the study design did not allow us to evaluate whether additional effects would have been observed if the same amount of fructose had been consumed as sucrose. However, several studies reported similar deleterious effects of both fructose and sucrose on lipid metabolism in humans when compared with glucose (10, 11, 36). Our study was also limited to a small group of healthy, young male volunteers. Whether the same results would have been observed in females, in older individuals, or in overweight or obese subjects clearly awaits further studies.

In conclusion, we showed that in healthy subjects, consumption of moderate amounts of fructose for 4 wk produced a sustained increase in fasting VLDL-triacylglycerol and a modest but significant rise in fasting glycemia. This increase in plasma VLDL-triacylglycerol, observed with a dietary fructose intake commonly encountered in westernized countries, may increase cardiovascular risk over the long term. Insulin resistance and ectopic fat deposition were, however, not observed. This leads us to propose that some of the deleterious effects of fructose may possibly be prevented in healthy subjects by adaptive metabolic changes in hepatic cells, skeletal muscle, or adipose tissue. Further studies will be required to evaluate the responses to HFD in subgroups of individuals with increased metabolic risk (such as offspring of patients with type 2 diabetes and overweight or obese patients) and the long-term consequences of a HFD in healthy subjects. 

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K-AL, DF, RS, and LT designed the study and performed the clamp experiments. MI, RK, PV, and CB performed the MR measurements. K-AL, DF, ER, and LT analyzed the data. K-AL, DF, and LT wrote the draft manuscript with contributions and critical reviewing from ER, RS, MI, RK, PV, and CB. All the authors read, commented on, and contributed to the submitted and revised manuscript. None of the authors had a conflict of interest.

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